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DNA Microarrays

A PRACTICAL APPROACH

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Confocal scanning microscopy in microarray detection

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1. Introduction: microarrays, fluorescence, and detection

All microarrays require fluorescence scanning to extract their experimental results. The confocal laser scanner delivers the highest image and data quality, a significant performance advantage. This chapter will describe the scanning process from an instrument point of view. It is divided into three parts. Sections 1 and 2 will describe the relevant characteristics of microarrays and all types of microarray scanners. Sections 3–7 will describe the design options and critical characteristics of confocal scanners. Finally, Section 8 will describe one commercial confocal implementation, the ScanArray®.

1.1 Detection characteristics of microarrays

This section will describe the aspects of microarrays that are most relevant from the point of view of a scanning instrument. Microarrays consist of small samples of DNA or other biological matter arranged on a flat surface. The DNA or other material is tagged with a fluorescent probe so that a fluorescence measurement will reveal the concentration of the sample, with a typical fluorescence dynamic range of between about 400:1 and 4000:1 limited by background fluorescence. The flat substrate is generally made of chemically treated glass, and is often in the form of a 25 mm × 75 mm microscope slide. Microarrays are described at length in several other chapters; this chapter will confine itself to the aspects of microarrays related to fluorescence detection.

Many DNA arrays incorporate samples tagged with multiple fluorescent probes, most often two. In the example of differential gene expression testing one probe is the control (normal tissue, for example) and the other probe represents the test (diseased tissue). The sample is scanned at two wavelengths, and the ratio of the fluorescence emissions of the two wavelengths represents the differential gene expression. This ratiometric approach reduces the need for absolute calibration of the sample preparation process. Array scanners generally require at least two different 'channels', or detection wavelengths.

A microarray consists of 'dots' and 'background', where the dots are the samples and the background is all of the area between the dots. While the substrate of the array is glass, the dots actually bind to chemical surface treatment on the surface of the glass. Surface treatments are used here to provide chemical binding affinity to the DNA samples and to produce very high surface tension. High surface tension prevents the liquid droplet of sample from spreading out when the array is made, helping to keep the dot size small and uniform, allowing increased density of the dots.

Microarray fluorescence data analysis almost always compares the fluorescent intensity of each dot to the local background around the dot. The substrate's surface treatment can degrade the scanning results if it fluoresces measurably in the wavelength ranges of interest and increases the fluorescence background level.

Dots may be anywhere from 25–500 μm in diameter. As of this writing, most DNA microarray dots are 100 μm ± 50 μm diameter, with the trend toward the smaller sizes. The dots are formed by small droplets of liquid drying in place on the substrate. Dot diameter variation affects scanning results. Scanners detect the area concentration of fluorescent dye; a droplet that spreads more than its otherwise identical neighbour will have a lower area concentration of dye, and will produce a scan signal that is inversely proportional to the dot area.

Contamination of the array by dust or almost any material can also affect scanning results adversely. All organic materials fluoresce. Microarray scanners that are sufficiently sensitive for the application will also clearly detect common airborne dust particles (e.g. clothing fibres, skin, finger oil).

Dry spotted microarray dots are quite thin, usually less than 10 μm. This thinness allows the use of the confocal scanning approach. Confocal scanning (described in Section 3) deliberately limits the depth of focus of the scanner, which prevents the imaging of many undesired background artefacts. Fluorescent dust, contamination on the back surface of the sample, fluorescence of the glass substrate itself, and fluorescence contamination from any of the scanner's internal optical components which may be 'glowing' in the field of view are all strongly attenuated by the confocal approach.

There may be significant variations in the fluorescence signal intensities between arrays, often due to minor differences in dye incorporation during reverse transcription and subsequent hybridization yields during microarray sample preparation. Microarray scanners need to have a sensitivity adjustment range that probably far exceeds the dynamic range of measurement of any one sample. A sensitivity adjustment range of at least 10 000:1 is required for a general purpose instrument.

1.2 A brief description of fluorescence

Fluorescence in biological detection is a broad topic that has been described more comprehensively elsewhere (1). A brief description highlighting the aspects most relevant to microarray scanning is offered here.

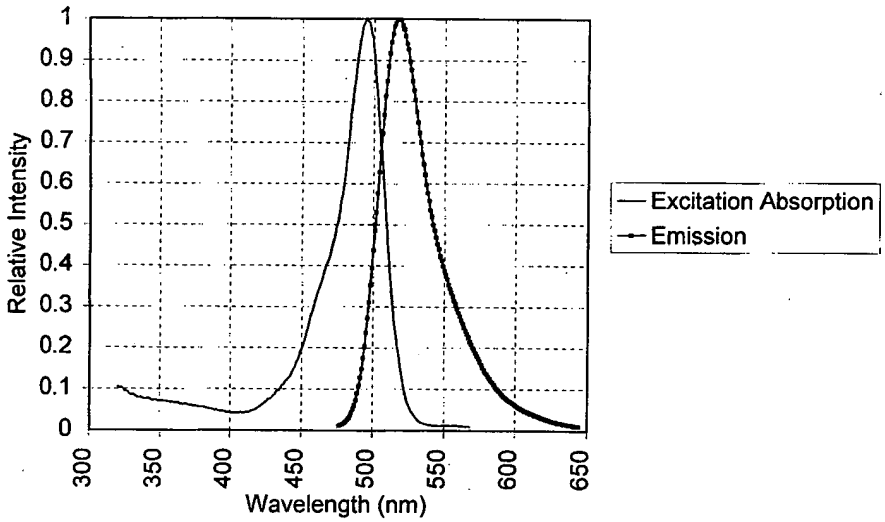

Figure 1. The relative excitation efficiency versus wavelength, or excitation absorption, for FITC (left-hand curve). The emission wavelength spectrum is also shown (right-hand curve) when the dye is excited at 400 nm.

Fluorescence light is emitted from a dye or fluorophore which is illuminated by excitation light. The fluorescence emission wavelength for the conventional fluorescence used in microarrays is always longer than the wavelength of the excitation light. Each dye has a curve describing the efficiency of excitation versus wavelength; an example curve for FITC (fluorescein isothiocyanate, a common dye used in microarrays) is shown in *Figure 1*.

This graph shows an excitation curve with a peak at 494 nm and fairly steep slopes away from both sides of the peak. The emission curve peaks at 518 nm. The wavelength difference between the emission and excitation peaks is 24 nm, which is typical for most dyes used in microarrays. This wavelength difference between the two peaks is called the Stokes shift.

Graphs such as these can be misleading at first look. This one suggests that, for example, that FITC can be excited at 510 nm, and produce some fraction of the fluorescence emission in the 475–510 nm range. That is not so; the emission is always at a longer wavelength than the excitation. The excitation curve and emission curves on this graph were not produced simultaneously. They are in fact very different sets of data placed on the same numerical grid for convenience of display.

The excitation curve is generated by measuring fluorescence emission at a single, long wavelength while the excitation wavelength is varied. The emission is measured at a wavelength longer than the longest excitation wavelength

intended to be characterized. In *Figure 1*, the fluorescence was measured at 600 nm while the excitation was varied to generate the excitation curve

The emission curve is generated by measuring the intensities of narrow bands of fluorescence at many different wavelengths while keeping the excitation wavelength constant. The excitation wavelength used is shorter than the shortest emission wavelength intended to be measured; it was about 350 nm in the case illustrated in *Figure 1*. The emission curve is a true spectrum. The emitted light is not all at one wavelength but is distributed about a peak.

Reading such a graph allows the microarray scanner designer to specify wavelength selection components appropriate for the given dye. Excitation light should be provided at a wavelength that provides reasonably efficient excitation, at least at the 50–70% level. The excitation wavelength cannot be too close to the emission peak or it will pollute the fluorescence signal, so it needs to be on the left side of the excitation peak. For FITC, that suggests excitation wavelengths between about 470–495 nm.

Fluorescence is generally linear, such that the power of the emission light is directly proportional to the power of the excitation light. This process breaks down at high levels of excitation where the fluorescence output saturates or the dye is damaged by the incoming photons. Also, the fluorescence emission is typically orders of magnitude weaker than the excitation light in microarray applications.

The final aspect of fluorescence emission that is relevant to microarray scanners is that the emission from a dye molecule is spherical. Each dye molecule absorbs excitation photons and emits fluorescence photons. The emitted photon can go in any direction, with no directional preference relative to the incoming light. A fluorescence detecting instrument must therefore gather in the emission light from some fraction of this sphere. As microarray scanning utilizes very low levels of emission, this geometric fact is a major driver of the instrument design.

1.3 Optical requirements of a detection instrument

Microarray scanners can take many forms, whether confocal or not, but any of these instruments must provide the following functions:

- excitation
- emission light collection
- spatial addressing
- excitation/emission discrimination
- detection

1.3.1 Excitation

Excitation light can be generated by a variety of sources, such as lasers, arc or filament lamps, or LEDs. Excitation must be limited in its wavelength so that

it does not overlap the emission, so broadband emitters such as lamps and LEDs require filters to select the excitation spectral band. Lasers generally produce light at a single, well-known wavelength (with some complications in diode lasers) and may not require excitation filtering. Lamp sources can be used to provide multiple excitation wavelengths with the simple addition of an excitation filter changer or other wavelength tuner. Multiple lasers are required in instruments delivering multiple laser excitation wavelengths. The function is sometimes provided by a multi-wavelength laser (also called multiline; laser wavelengths are referred to as laser lines), but these tend to be more awkward and expensive than multiple lasers.

The excitation light must be directed onto the microarray sample. This can be done in a flood illumination manner, where a large area of the sample is excited at one time. Flood illumination is most often used with CCD camera type instruments and is infrequently used with confocal scanners. Non-uniformity of flood illumination across the excitation area on the sample is a concern that the instrument designer must address when using this method.

Alternatively, the excitation light may be focused to a small spot to illuminate a very small portion of the sample. This choice is tied in with light gathering and spatial addressing, described below. Focused spot illumination provides very intense excitation (up to 10 000 watt/cm^2 or more in microarray scanners) for a very short time on a given area as the spot scans across the sample.

Excitation wavelengths are chosen based on the intended dyes, as described in Section 1.2. The wavelength should be on the left side of the dye's excitation peak, but still in the range where the excitation efficiency is good. The lower the excitation efficiency, the more excitation light must be delivered to the sample to provide a given level of fluorescence. Excessive excitation light is not desirable; it can either damage the sample through photobleaching or pollute the fluorescence emission signal, which is much lower in intensity.

1.3.2 Light collection

The fluorescence light is most often gathered or collected with an objective lens. This is a lens that focuses on the sample and directs all emitted light within some angular range or cone into a detection path. The size of the solid angle of collection is critical. As the fluorescence emission is spherical, the fraction of the sphere that is intercepted by the objective lens is the first determinant of the light gathering efficiency of the instrument. The light collection angle of a lens is most often characterized by the numerical aperture (or NA). *Figure 2* is a plot of numerical aperture versus light collection efficiency for a point source spherical emitter.

An NA of 1.0 describes a lens that collects light over an entire hemisphere; this corresponds to a collection efficiency of 50%. Most confocal laser microarray scanners have NAs between 0.5–0.9, while most CCD-based array scanners have NAs between 0.2–0.5.

Still other array scanners do not use objective lenses. An integrating sphere

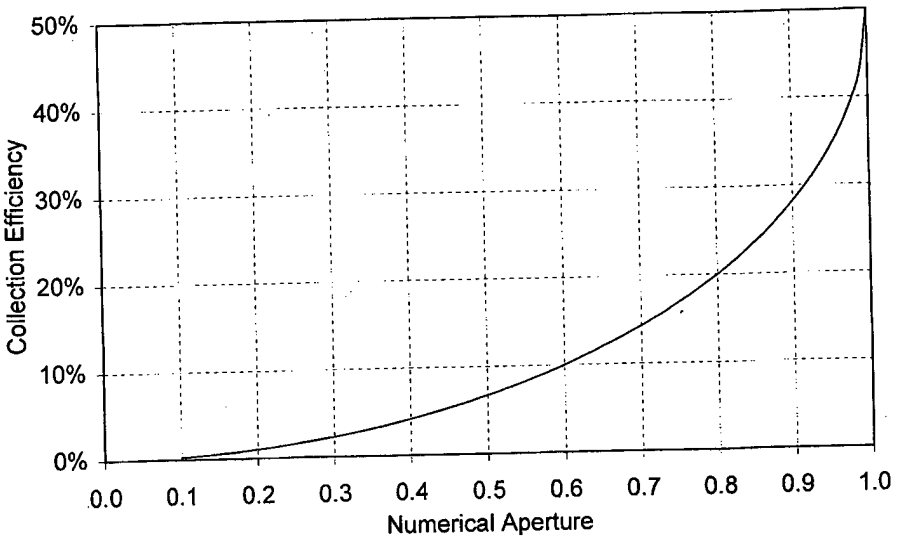

Figure 2. Collection efficiency of an objective lens at various NAs.

may be used to gather some fraction of the emission light, although with efficiency diminished from multiple reflections in the sphere (2). Yet another approach is to use no explicit light gathering element at all, and simply placing a detector with some area in a staring position over the sample. This arrangement's light collection efficiency is determined by the solid angle subtended by the detector compared to the sphere defined by the illuminated point on the sample. For example, some breadboard scanners utilize a bare 25 mm diameter detector about 100 mm from the excitation point, yielding an effective numerical aperture of 0.12.

1.3.3 Spatial addressing

Spatial addressing refers to measuring the fluorescence from small, specific areas on the sample. The sample is divided up into pixels, where each pixel is significantly smaller than a microarray dot. The spatial resolution must be finer than the dot size so that dot-edge artefacts and other non-uniformities can be accounted for in the quantification of the fluorescence signal. Scanners for 100 μm diameter microarray dots commonly use pixel sizes between 5–20 μm.

Spatial addressing is done either by using a multi-element detector array, such as a CCD, or by mechanical scanning. Most CCD cameras are configured to stare at an area that has been flood illuminated, and provide an image divided into pixels directly by the CCD detector. The limitations to this approach are the generally smaller NA lenses accommodated by CCD

detectors and the cost of the back-illuminated, actively cooled CCD elements, and cross-talk between pixels due to optical scatter.

Mechanical scanning involves focusing the excitation beam to a point about the size of a pixel, and collecting emissions from just that small area with a single element detector. To cover the area of the sample, motion or scanning is required. This can be done by scanning the beams with mirrors (3), moving the sample, or a combination of both. Scanning adds considerable mechanical complexity to the system compared to a CCD camera system, but allows the use of higher NA light collection optics, higher spatial selectivity, and less expensive detectors. In low light applications, the higher optical collection efficiency is paramount.

1.3.4 Excitation/emission discrimination

Microarray fluorescence emission power is typically several orders of magnitude less intense than the excitation power. In order to detect the small fluorescence signal without a large contribution from the excitation light, some optical means must be incorporated into a microarray scanner to separate the two types of light. Since the beams are at different wavelengths, wavelength separation is the primary means, although specific geometric arrangements contribute as well.

Most objective lens-based microarray scanners use epi-illumination, where the excitation and emission beams follow the same path through the objective lens to and from the sample, but in opposite directions. This arrangement allows reflections and scatter from the sample to mix with the fluorescence beam. A beamsplitter element is used as the first separator of these beams.

One type of beamsplitter is a colour separating dichroic or multichroic interference filter, which reflects the excitation beam and transmits the emission beam at a slightly longer wavelength. These are commonly available, and can work well with one, two, or three different excitation/emission wavelength pairs. With four or more wavelengths, it is difficult to design and make a single multichroic beamsplitter with good performance at each colour.

A different type of beamsplitter is a geometric beamsplitter, shown in *Figure 3*. In scanning systems with an objective lens NA above about 0.6 and approximately 10 μm pixel size, the excitation beam is much smaller in diameter than the emission beam upstream of the objective lens. A small mirror, which reflects the laser beam but passes the bulk of the emission beam in an annular section works well as a wavelength-independent beamsplitter.

A beamsplitter that worked perfectly would complete the excitation/emission separation task, but no beamsplitter works perfectly. Emission filters are typically placed in the emission beam before the detector. These are interference filters that pass a narrow band of wavelengths near the dye's emission peak, and block all other light including the excitation light. This second layer of discrimination is required in the microarray application.

Scanners are built without beamsplitters by placing the excitation and

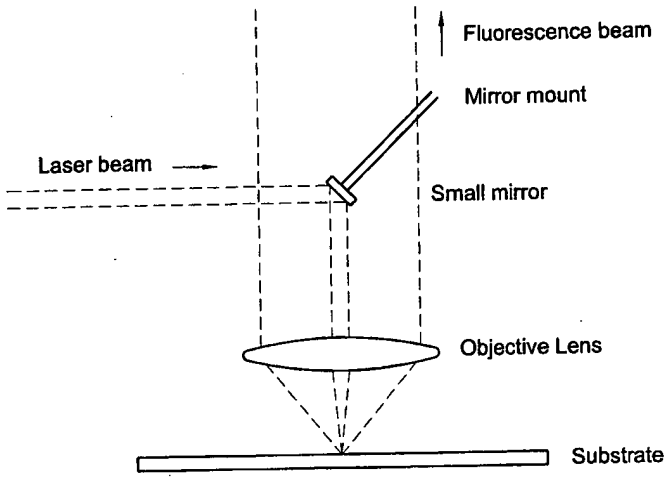

Figure 3. A geometric beamsplitter in an epi-illuminated scanner.

emission beam paths on different axes. This approach can work well for rejecting excitation light from the emission path, but it is difficult to implement with a high NA objective lens. Such lenses need to be placed very close to the sample, often less than 1 mm away, leaving little room for an excitation beam to enter from an angle.

Other types of components can be used for wavelength discrimination, such as prisms and gratings. These can offer some interesting features, such as continuous wavelength tunability. However, properly specified emission filters generally perform the best and most cost-effectively in microarray scanning due to the high excitation light rejection required.

1.3.5 Detection

The detector in a fluorescence scanner converts the low levels of light to an electrical signal. Detectors found in array scanners include photomultiplier tubes (PMTs), CCD arrays, and avalanche photodiodes (APDs). Each has advantages and disadvantages, and must be selected as part of the overall architecture of an instrument for a particular range of applications.

PMTs are the most sensitive detectors in the visible wavelength range. They are single point detectors and require a scanning system to provide spatial addressing of a microarray sample. It is convenient to change the PMT sensitivity by varying a control voltage, which provides at least part of the sensitivity adjustment range required in an array scanner. PMT sensitivity falls off rapidly between the red and near-IR, so their usefulness is generally limited to the visible range.

A CCD doesn't have the inherent low noise amplification of a PMT, and therefore usually needs more external amplification to reach the maximum

sensitivity of a PMT. One major disadvantage is the difficulty in using a CCD detector with a high NA (0.6–0.9) collection system, limiting the optical signal available for detection. This is very important in dim light situations, as the statistics of the photon stream will ultimately determine the detection floor of the instrument. The other disadvantage is the impracticality of incorporating a CCD in a confocal system, the advantages of which are detailed in Section 3. In higher light applications, these disadvantages may be outweighed by the CCD detector array's built-in scanning mechanism.

2. Sample handling

Since all microarray scanners need to hold and locate the array substrate so that it is positioned properly with respect to the optics, this topic applies to all types of array scanners. This aspect of an instrument often requires precision positioning to maintain constant focus and image geometry from sample-to-sample. This section will mostly refer to microarray substrates that are glass microscope slides, which constitute the vast majority of spotted microarray substrates used today. Most of the considerations discussed apply equally to non-standard proprietary substrates as well.

2.1 'Standard' microscope slides

Microscope slides have become the *de facto* standard substrate for microarrays and dominate the market, but there is no single standard describing microscope slides. They are widely available in a variety of sizes, thicknesses, edge and corner configurations, and end treatment. *Table 1* summarizes the selection available from just one large American laboratory supplier, with some dimensions and tolerances. A general purpose scanning instrument needs to be able to accommodate the full range of slide types.

Table 1. Some characteristics of 'standard' microscopic slides

Slide type	**'Inch': 1" × 3"**	**'Metric': 25 mm × 75 mm**
X-Y dimensions, inches (mm)	1.0" × 3.0", ± 0.02" (25.4 mm × 76.2 mm, ± 0.5 mm)	0.98" × 2.95", ± 0.02" (25 mm × 76 mm, ± 0.5 mm)
Thickness dimensions	'Standard': 0.04" ± 0.002" (1.02 mm ± 0.05 mm) 'Thick': 0.047" ± 0.004" (1.2 mm ± 0.1 mm)	0.04" ± 0.002" (1.02 mm ± 0.05 mm)
Corners	Sharp, bevelled	Sharp, rounded
Edges	Sharp, bevelled	Sharp
Surface treatments on ends	Plain, sandblasted, painted; one or both sides	Plain, sandblasted, painted; one or both sides

Locating the sample by its top surface simplifies the accommodation of large thickness variations. The sample holder should allow easy loading and unloading of a sample, and may need to accommodate an autoloading accessory for high volume scanning. The materials used in the sample holder must withstand the abrasion of thousands of insertions and removals.

2.2 Other formats

Other sample formats in development and limited use as microarray substrates include plastic slides, proprietary glass substrates of various shapes and sizes, and enclosed liquid-filled cells. Each requires specific sample holding solutions; for example, plastic is much less stiff than glass and more challenging to keep flat and in-focus in a confocal scanner. Liquid-filled cells require imaging through the glass to the second surface which typically requires a specific objective lens design as well as a custom sample holder.

2.3 Sample environment

Most microarrays are scanned at room temperature in the dry state, with the dots dried on the surface of the substrate. Some popular dyes, FITC for example, emit stronger fluorescence in a wet state. This condition is produced in a scanner by wetting the array with the proper buffer solution and covering it with a thin cover glass. The sample holder must accommodate the full range of coverslip sizes to be useful in these applications. The scanner then scans through the cover glass.

Some researchers are investigating the scanning of arrays while controlling and varying the temperature while scanning to reveal additional information about the sample. At this writing, this capability is not available in any commercial scanning instrument.

3. Confocal scanning of microarrays

The above sections were a preamble to the main topic, which is the 'how' and 'why' of confocal scanning. This section will describe the confocal scanner optical arrangement, showing how the confocal arrangement blocks out many unwanted image artefacts, and discuss its advantages and disadvantages.

3.1 The confocal arrangement

With the above background in place, this section will illustrate the confocal scanning architecture and discuss its tradeoffs. As the name implies, confocal scanners have two focal points configured to limit the field of view in three dimensions. This limits light gathering from all locations outside of a small volume and rejects a variety of image artefacts. Confocal systems by definition image only a very small area (effectively one point; one pixel) and require scanning to acquire a multi-point image from a two-dimensional surface.

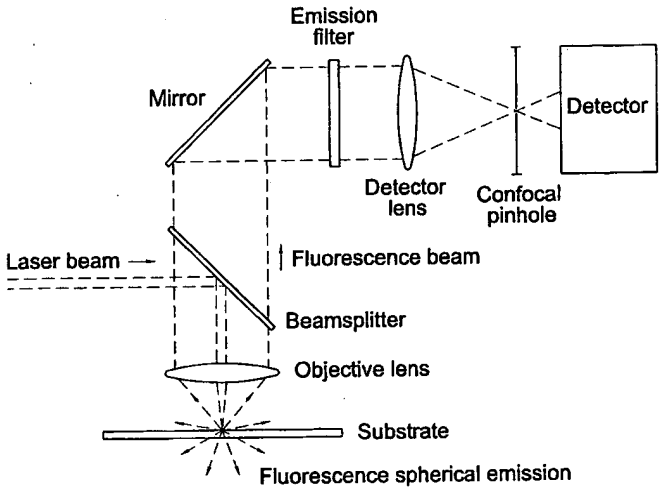

Figure 4. The confocal scanning arrangement in a microarray scanner.

The confocal optical arrangement shown in *Figure 4* operates as follows. The collimated (or parallel) laser beam is reflected from the beamsplitter into the objective lens. The laser beam fills only a fraction of the lens; how much depends on the particular choice of lens NA and the desired pixel size. The laser beam is focused on the sample, where it induces spherical fluorescence in all directions. The excitation beam also reflects back up toward the detector.

The objective lens collects a fraction of the spherical fluorescence emission and collimates it into a parallel beam. It also collects the reflected laser light, which is three to seven orders of magnitude more intense than the fluorescence. This return beam is again directed to the beamsplitter, which reflects most of the laser light back toward the laser source, and transmits most of the fluorescence beam up toward the detector. A mirror then folds the system without any optical functionality, followed by the emission filter which selects a narrow band of fluorescence emission and rejects all remaining laser excitation light.

The confocal nature of the system is embodied in the detector lens and the pin-hole. The detector lens focuses the collimated fluorescence beam to a small diameter, and a pin-hole is placed to allow passage of that focused beam and block all other light. This has the effect of restricting the depth of focus of the objective lens. A look at the path of an out-of-focus image through *Figure 5* reveals this.

A second point source of light below the focus on the sample, caused by a piece of dust for example, would emit light that also enters the objective lens. Since this second point is not at the objective lens' focus, however, the fluorescence beam travelling up toward the detector is not collimated (parallel), it

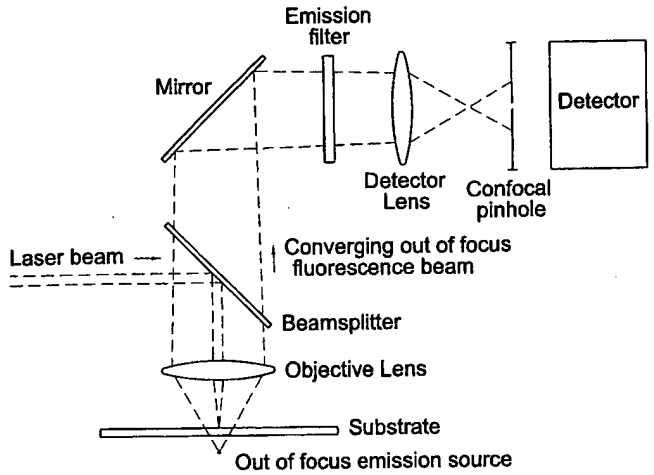

Figure 5. Emission light from an out-of-focus image point is blocked by the confocal pin-hole.

is converging. When this converging beam passes through the detector lens, it forms a focus in front of the pin-hole instead of at the pin-hole. The beam expands after the focus and has a large diameter where it strikes the pin-hole. This causes the pin-hole to reject most of the out-of-focus light. The same pin-hole rejection effect happens with light sources above the objective's focus. Only light from a narrow depth of focus at the sample can pass through the pin-hole efficiently.

3.2 Advantages and disadvantages of the confocal arrangement

In a microarray scanner, this restricted depth of focus strongly reduces the imaging of artefacts on the sample, such as dust and rear-surface reflections from the substrate. It also nearly eliminates the instrument's sensitivity to unintended fluorescence of components within the instrument, such as filters or the sample holder. In practice, it produces microarray images and data with dot-to-background (signal-to-noise) ratios far superior to non-confocal instruments.

The chief disadvantage of the confocal system is also the restricted depth of focus. It requires the scanning motion that generates the image to be performed with a deviation from flatness that is much less than the depth of focus, on the order of $\pm$ 10 μm. In systems where the beam is scanned, the field-flattening or F-theta scan lens must produce a very flat field. In all systems, scanning beam or moving substrate, the substrate must be kept flat and the substrate motion must also be kept flat. This complicates the mechanical and optical design but is the preferred path to maximum image quality.

3.3 Confocal implementations and tradeoffs

The confocal arrangement as shown in *Figure 4* is appropriate for a moving-substrate scanner. The arrangement can be implemented in a moving-beam scanner by inserting a scanning mirror between the beamsplitter and the objective lens (as in ref. 3), and making the objective lens a flat-field scan lens. A scan lens accepts a beam scanning through various angles and focuses it along a flat, straight line. Such lenses that can scan the width of a microscope slide are quite complicated and are generally limited to a NA of about 0.3 or less.

Simple objective lenses and much higher light collection efficiencies can be obtained with moving-lens or moving-substrate systems. A moving-substrate instrument moves the substrate in a raster fashion under the stationary optical system. A moving-lens system moves an entire optical head over a stationary substrate. The tradeoff is that these systems with larger moving masses typically have lower maximum scanning speeds than moving-beam systems. However, the high scanning speeds of a moving-beam system may not be usable in dim light situations where the statistics of photon detection determine the detection floor, so the gaining of potential scanning speed with the reduction of light collection efficiency can be self-defeating. Kain *et al.* (4) describe a moving-lens system, and Section 8 describes a moving-substrate system which takes advantage of the simple lens with high NA.

4. Wavelength discrimination: minimizing the background image

This section will describe in greater detail the instrument design criteria and tradeoffs involved in wavelength discrimination, or separating the desired fluorescence light from all of the other light before detection and quantification. The usefulness of the instrument to a bioresearcher hinges on this capability to a large extent. While the principles outlined here apply to all types of fluorescence scanners, the most desirable implementations for maximizing image quality can only be implemented in the laser confocal type.

Useful microarray scanners must detect very low levels of fluorescence light, in the picowatt range. At this low level, almost all materials fluoresce: the glass substrate, the chemicals comprising the substrate's surface coating, sample washing chemicals, lenses, filters, even DNA itself. The scanning instrument needs to maximize detection of the target dye's emission while minimizing detection of all of these other fluorescence sources. In addition, the reflected and scattered laser light, which is about one million times brighter than the dim fluorescence light from the microarray, must be rejected as well. Failure to separate the dye emission light properly from all of the competing sources results in a high background signal level. This background

signal can define the dye concentration detection floor of the scanner and needs to be minimized.

4.1 Beamsplitter

All confocal scanners have some type of beamsplitter, as shown in *Figure 4*. This component must separate out the returning beam from the sample from the excitation beam. The glass sample substrate reflects about 4% of the laser light from the first surface. Some researchers are investigating the use of mirrored sample substrates to increase the fluorescence returned back up the optical path. A mirrored substrate reflects nearly all of the laser light.

The beamsplitter should reject most of the reflected laser light from the detection path while passing most of the fluorescence. A common component used for this is a dichroic or multichroic interference filter. These components commonly exhibit reflectance of over 90% for one, two, or three laser lines, and transmission in the 60–85% range for one, two, or three fluorescence bands. The 90% laser line reflection eliminates all but 10% of the laser reflection from the detector path. These components work adequately in many applications, but become increasingly difficult to implement with larger numbers of laser and fluorescence wavelengths in the instrument.

A geometric beamsplitter, such as the one illustrated in *Figure 3*, can show markedly improved performance over the dichroic type, with the added benefit of wavelength independence. Implementation of such a beamsplitter requires a number of instrument optical parameters to all be selected in concert. The primary assumption for the use of such a splitter is that the collimated excitation beam must be much smaller than the returning emission beam. This can only happen when the choice of focused excitation spot size and the focal length and NA of the objective lens allow it. In general, the technique works better with higher NAs. With an NA of 0.75, a focal length of 6.6 mm, and an input laser beam diameter of 0.75 mm, an objective lens aperture of 10 mm, and a focused excitation spot size of about 6 μm, the technique works quite well. The geometric splitter for this system can be constructed to transmit about 80% of the emission beam while rejecting almost all of the reflected laser light. This type of beamsplitter has been shown to increase the signal-to-background ratio on very dim samples by a factor of three over a dichroic splitter.

Other types of beamsplitters, such as polarizing beamsplitters and 50/50 broadband splitters, do not have sufficient excitation light rejection to be effective in microarray scanners.

4.2 Emission filters

The beamsplitter in a confocal microarray scanner is not adequate by itself for discriminating the fluorescent dye signal from the other light sources. As shown in *Figure 4*, an emission filter can be placed in the collimated emission

beam prior to the confocal detector lens. An emission filter should transmit the desired wavelength band of the fluorescence signal and block all other light.

Examining, as an example, the fluorescence emission of FITC in *Figure 1*, the reader might conclude that the best emission filter would be one that includes most of the emission curve in its passband, say from 510–600 nm. This would deliver the maximum fluorescence light to the detector. In practice, the light in the emission beam is a more complicated mix. In addition to the dye's emission curve, there are several other emission curves competing with it: fluorescence of the glass substrate, the surface preparation chemicals, the sample washing chemicals, etc. These other 'noise' fluorescence curves are in general not defined and are variable. With that, the emission filter needs to maximize the dye signal while minimizing the competing fluorescence.

The best way to do that is to use a narrow band emission filter with the passband centred on the dye emission peak, as shown in *Figure 6*. This figure shows a filter passband that is 10 nm wide at its half-intensity points (FWHM). Passing the dye emission at its maximum intensity in a narrow window while blocking all light outside of the passband maximizes the signal-to-noise. The penalty for this approach is low throughput of the signal. The 10 nm FWHM, six cavity interference filters which have been found to provide optimum signal-to-noise performance typically have about 60% transmission at the peak, and as *Figure 6* shows, only a fraction of the total dye emission spectrum

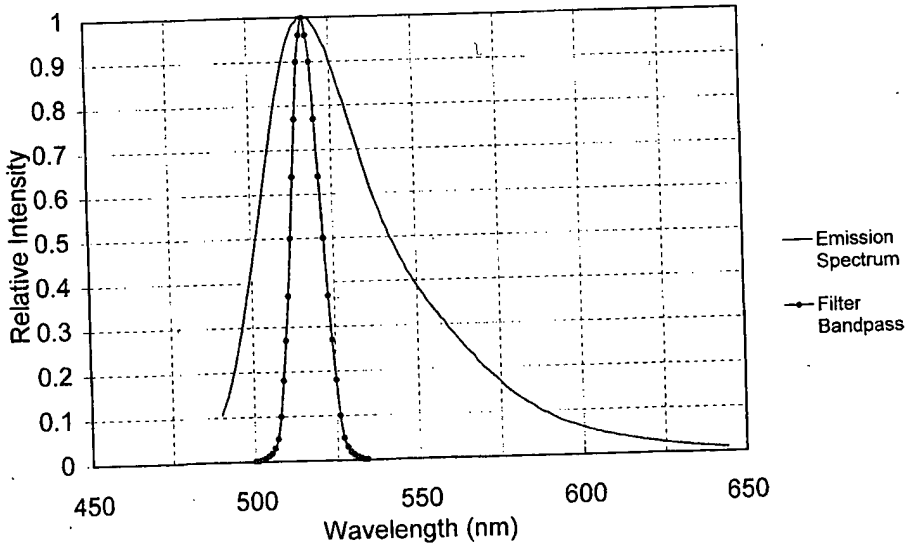

Figure 6. Pictorial superposition of a 10 nm FWHM bandpass emission filter on FITC emission.

overlaps the passband window. Such a filter will typically transmit 10–15% of the total dye emission.

The low optical throughput of a narrow band emission filter can only be tolerated in an instrument with high collection efficiency; e.g. a high NA objective lens. A microarray scanner with 10 nm FWHM emission filters centred on the dye peak works well with NAs above about 0.7. Filters with wider passbands increase background transmission more than signal transmission, and narrower filters simply bring signal and noise down together.

Emission filters also need to block the residual laser light not stopped by the beamsplitter. Conventional narrow band filters typically provide about 10^4 attenuation of the excitation wavelength 20–30 nm down from the dye's emission peak. Microarray detection performance is enhanced by greater attenuation at the specific laser wavelength. Either custom emission filters specified to block the laser line or separate laser blocker filters that increase laser attenuation to about 10^7 produce measurable performance gains.

5. Detectors

Confocal laser microarray scanners use PMTs as detectors almost universally. In spite of the apparent disadvantages of tubes (the need for high voltage, large unit-to-unit variation in sensitivity, larger size than photodiodes), the advantages dominate in most visible light microarray scanning applications.

A PMT can detect a single photon, or a beam of light that is so low in power that it is a series of photon events rather than a continuous power flux. The amplification built into the PMT amplifies the photon events into electron events with amplification factors of about one million, producing a low noise current which is straightforward to filter, integrate, and quantify using conventional instrumentation electronics. In addition, the PMT sensitivity or gain can be adjusted through a range of several hundred to one by varying the tube high voltage input. This built-in wide range of adjustment is ideal for microarray scanning where variations in the sample preparation process dictate that requirement in the instrument.

Conventional silicon detectors, such as PIN photodiodes, don't have the built-in low noise gain of a PMT, and also have significantly lower quantum efficiencies in the visible wavelength range. Thus, they require external amplification of several million to reach the equivalent light-to-signal sensitivity. This external amplification adds noise, resulting in a lower signal-to-noise ratio. Silicon detectors have a wavelength response that is peaked in the 800–900 nm range, which makes them more attractive in the near-IR range. PMTs typically show peak sensitivity in the 500 nm range and start falling off rapidly beyond 650 nm.

Avalanche photodiodes (APDs) do have built-in, low noise gain similar to the PMT. However, they still provide much lower quantum efficiency in the visible range and lack the PMTs convenient sensitivity adjustment mechanism.

Researchers have used cooled avalanche photodiodes successfully in array scanners utilizing near-IR dyes, but PMTs are superior for the more common visible dyes.

6. Signal processing and instrument control

The previous sections have focused on sample handling and particularly on optics and detection. While these disciplines reside at the core of any microarray scanner, the instrument is not complete or of use to a bioresearcher without signal processing and means for controlling the scanner. This section will illustrate the control and processing functions required in a complete instrument.

6.1 Sensitivity range requirements

The fluorescent yield of any microarray sample is largely unknown until it is scanned. Sample preparation steps that have high variability include dye incorporation during reverse transcription and hybridization. Different types and batches of gene expression arrays using the same dyes can exhibit brightness differences of 1000:1 from one to another. Well prepared samples can exhibit dynamic range of fluorescence of up to about 4000:1 within the microarray. Since the dynamic range of most scanners spans from about 4000–16 000, there isn't enough dynamic headroom to accommodate the widely varying samples without changing the instrument sensitivity.

Two techniques are used to adjust sensitivity. First, the excitation power can be varied, typically by using a variable laser attenuator. Excitation power can usually be varied over a range of at least 100:1. Secondly, the PMT sensitivity can be changed, also over a range of at least 100:1, by varying the high voltage input. Both techniques superimpose, so an instrument adjustment range of more than 10 000:1 can be obtained by incorporating both. This is adequate for a general purpose instrument.

Using as much excitation power as possible without damaging the sample by photobleaching is generally desirable. More excitation power generates more fluorescence photons, generating a cleaner and statistically more robust signal at the beginning of the imaging chain. If a sample is to be scanned many times, the laser power may need to be dropped to lessen accumulated photon damage to it.

Since these instruments have such a large adjustment range, it can be difficult to find the proper operating point without many trial scans. An automatic routine in the instrument that sets the sensitivity to make the brightest image feature just short of instrument saturation saves the user a lot of time.

6.2 Signal averaging and sampling

The optical signal generated by the excited fluorescent dyes is processed for conversion into a sequence of digital values. The signal consists of a stream of

random photon emission events that, when properly averaged, correlate to the area density of dye molecules. However, the photons are emitted randomly over the space within a pixel and over the time of sampling. Therefore, the scanning instrument averages both spatially and temporally.

The scanned region is divided into equally sized pixels. Confocal and other spot-illuminated instruments independently excite each pixel and the resulting emitted fluorescence is collected for detection. The laser spot acts as a moving spatial averaging mechanism since the emission from the total spot area is summed during detection.

The shape and size of the laser spot determine its averaging characteristics. The spot typically has a Gaussian intensity profile that results in the dye molecules illuminated by the centre of the spot to be more excited and to emit more, and therefore to be more heavily weighted in the averaging processes. The FWHM spot size is typically chosen to be equal to that of the pixel. Larger spots would tend to average pixels together and smaller spots would not include the complete pixel in the spatial averaging.

After the optical signal is detected and converted to an electrical signal by the PMT, it requires further processing before being converted to digital data. The electrical signal still contains the randomness associated with individual photon emission events over time. The digitization process can be considered instantaneous relative to pixel dwell time. Electronic filtering is used to average the photon emission events into a signal that represents the information contained in each pixel.

Typically a time averaging, low pass filter is employed that uses an exponentially decreasing weight for signal values prior to the instant of digital conversion. These filters produce an exponentially decaying signature when filtering a step function. The time constant of the filter—the time required for the filter to decay to about one-third of its initial value after a step change—is the parameter set for proper data conversion. In practice, the time constant is set between one- and two-thirds of a pixel period. Longer time constants tend to average pixels together. Shorter time constants will tend to allow single photon events to distort the final data.

6.3 Dark signal

The detection components produce a non-zero signal even in the absence of light. This is called the dark signal, and its contributors include the PMT as well as its subsequent amplifiers and other circuitry. To the extent that this signal is constant over the time of an image scan, it can be subtracted out of the signal, and then will not affect the dynamic range of the instrument. Components of the dark signal that vary during scanning do limit it.

6.4 Image acquisition, display, and storage

A confocal microarray scanner is intended to perform an area scan of a sample and provide a digital map of the fluorescent intensities of each pixel.

The scanning system, whether it moves the beam, or the sample, or some combination, must produce a raster scan over an area. For a general purpose instrument the scan area should be selectable, so that the user need not wait for the instrument to cover the entire sample when the area of interest is smaller than that. In addition, a preview high speed, low resolution scanning mode is desirable, scanning only every five lines or so just to find a small array in a large field quickly.

Digitization of the pixels should be at 16-bit intensity resolution. 16-bit is a convenient format for downstream processing and manipulation. As an image is being scanned, it should be displayed on a monitor for preliminary inspection to make sure the sample is the right one, the settings are correct, etc. Image display is most often done with the fluorescent intensity mapped into false colours so that the human eye can distinguish more discrete levels on the screen.

After the image is scanned, it is saved or transferred to another computer application for quantification or other post-processing. The 16-bit TIFF (tagged image file format; .tif) format is the *de facto* standard for transferring microarray images, although some proprietary systems use proprietary formats.

6.5 Control interface and automation

All of the above optical and electronic functions have the most value in the laboratory when the user doesn't need to think about them. The instrument becomes useful when it has a simple, complete, and intuitive user interface to control it, and when commonly used sequences are automated. Microarray scanners are always controlled from a computer console. The successful commercial scanners available today, which have been refined with a great deal of user feedback, utilize graphical user interfaces that allow a new user to become productive very quickly with very little training or reading of manuals. They also allow experienced users to maintain a high level of productivity with minimum distraction from details.

Automation is particularly valuable in saving time for the user in the following areas:

(a) Setting, saving, and recalling sets of scanning parameters for particular users and types of samples.

(b) Automatic scanning of the sample at two or more wavelengths, with automatic saving of the resulting image files.

(c) Automatic sensitivity setting on one channel (auto-ranging) by scanning at low resolution and automatically adjusting excitation and detector settings to maximize dynamic range.

(d) Automatic sensitivity setting on multiple channels, commonly called 'balancing the channels'.

(e) Automatic internal calibration of excitation power settings and detector sensitivity settings.

The scanner or its host computer should also provide multi-gigabyte data storage, network connectivity, and support for large capacity removable storage media.

7. Instrument performance measures

Microarray scanners are complex instruments, embodying a large number of parameters and specifications. This section outlines the most important performance measures that most likely determine the suitability of a particular instrument for an application.

7.1 Number of lasers and fluorescence channels

The number of available scanning wavelengths (colours, channels) is fundamental to the instrument. A single excitation laser may excite several dyes and be used with several emission filters. However, there will be significant 'cross-talk' between these multiple dyes if they are used on a single sample. Independent channels, ones for multiple dyes that can be used on a single sample, almost always require separate laser sources of different wavelengths. One exception to this is scanning done with energy transfer dyes, which utilize the emission from a first dye to stimulate a second dye; but this is outside of the mainstream of microarray scanning. Two or four fluorescence channels with two lasers are common in first generation scanners, and three or four lasers with up to 10 selectable fluorescence bands are available in second generation instruments.

7.2 Detectivity

Detectivity is the minimum dot fluorescent brightness that can be distinguished from the background when the sensitivity is set so that the brightest element of the sample produces an intensity level at the full scale. From an instrument point of view, its unit of measure would be the number of dye molecules per unit area (fluors/μm^2). Bioresearchers generally don't know what the dye density is, due to the sample process uncertainties, and view this measure from an application point of view, such as a threshold gene expression level. Detectivity is often characterized with specially made microarrays that incorporate dilution series: sequential array dots with systematically decreasing dye concentrations. The detectivity for that array preparation process is then defined by the dimmest dot in the dilution series that can be detected.

Either the sample or the instrument can place the limit on detectivity. If the sample preparation results in relatively high background fluorescence between the dots, the sample will be the limit. If the instrument injects noise into the signal that can't be subtracted out, at a level that exceeds the sample background, the instrument will be the limit.

7.3 Sensitivity

Sensitivity is the instrument's conversion efficiency of light power to a digital value at a particular wavelength. It is a measure of the 'gain' of the instrument. Unlike detectivity, sensitivity is not dependent on the characteristics of a particular sample. Sensitivity can limit detectivity only if the scan data from a sample is too dim when the excitation and detector are both adjusted to maximum values. More often, the maximum settings for the instrument are not used, as those settings produce saturated data values.

7.4 Cross-talk

When scanning samples with multiple dyes, cross-talk can occur. Cross-talk is the excitation and detection of a dye with the 'wrong' or unintended excitation wavelength and emission filter. In differential gene expression, cross-talk negatively distorts the expression ratio between the two channels. It is minimized by the use of narrow band emission filters centred on the dye peaks, with good attenuation of out-of-band wavelengths. It is most completely minimized by the selection of dyes and laser wavelengths which are sufficiently far apart (approximately 50 nm is a good rule of thumb) to allow proper filtering.

7.5 Resolution

The spatial resolution of a microarray scanner is usually expressed as a pixel size, with 5, 10, and 20 μm being common in commercial devices. It is important that each microarray dot be imaged into many pixels so that edge effects and other defects can be rejected at the quantification stage. As a rule of thumb, the pixel dimension should be no larger than 1/8 to 1/10 of the diameter of the smallest microarray dot to be imaged.

7.6 Field size

The field size, the area on the substrate that can be scanned, must match the array making process. The larger the scan area, the more dots that can be placed on each sample. Usually, a 1.0 to 1.5 mm border around the periphery of the slide is not used, as it may be chipped or not flat. Combined with the slide size tolerances outlined in *Table 1*, the maximum usable area is about 22 mm × 73 mm. Many commercial scanners only scan 60 mm in the long direction, assuming that the user will want a 15 mm 'handle' on the end of the slide for handling and labelling.

7.7 Uniformity

Uniformity is a measure of the consistency of fluorescence emission and detection across the field. Uniformity of light collection throughout the image field is of particular concern for confocal scanners. The confocal feature limits

the depth of focus, often to just a few tens of microns. This means that deviations from flatness in the scanning motion or in the sample itself at this small level will result in non-uniformity of the image across the field. Increasing the depth of focus by enlarging the confocal pin-hole relaxes the sample flatness requirements, but at the expense of adding unwanted image artefacts. Confocal scanners, particularly the moving-sample type, do have an advantage over flood illumination scanners in the delivery of uniform excitation power throughout the field.

Most users seek a ± 10% scanner uniformity across the field. At this level, sample preparation effects dominate the overall uniformity result.

7.8 Image geometry

Requirements for the scanner's image geometry depend greatly upon the post-processing applied to the image data. Image size, X-Y orthogonality, and pixel placement linearity matter a great deal if the image quantification software applies a fixed grid to the scanned image and expects to find dots in the centre of each box. More sophisticated microarray image quantification software applies a dynamic grid, 'finding' each dot regardless of its placement. Obviously, great precision in scanning geometry is less valuable if the precision delivered by the instrument that places spots on the array is low.

Most users are satisfied with image size and linearity tolerances of ± 2%. Many second generation scanners far exceed that level of precision.

Random geometry errors can also be important. These are often called 'jitter', and are manifested as jagged vertical lines in the raster image. When the line-to-line non-repeatability of scanning exceeds a pixel, the dot image's edges become jagged. This can cause problems in quantification when there are only a few pixels per dot.

The registration of multiple images of the same sample scanned at different wavelengths is also worth noting. Post-processing software that 'finds' the dots and automatically constructs a grid on the image usually creates the grid on the image from one channel and then uses the same grid on the images from different channels. Most array scanners maintain registration between subsequent scans at one pixel or less, which is adequate.

7.9 Throughput

Once all of the above image measures are proven adequate, users often focus on how many samples can be scanned in a day. Throughput specifications are only meaningful when applied to a resolution, an image field size, and the number of channels to be scanned on each sample. Some CCD camera-based scanners exhibit very high throughput, but are several factors less effective in detectivity of dim samples than even first generation confocal scanners.

Throughput for multichannel scanning can be increased dramatically by incorporating colour separating beamsplitters in the emission path, multiple detectors, and multiple signal processing modules and scanning multiple colours

simultaneously. However, this increases the scanner cost and complexity, especially for multiple wavelength instruments, and may increase optical cross-talk.

A typical specification for a first generation scanner scanning a single colour over a 20 mm × 60 mm field is 5–15 minutes at 10 μm resolution. Most second generation scanners are faster.

7.10 Superposition of signal sources

This is not a performance measure, but is an important concept that fits better at this point in the chapter than elsewhere.

The image viewed on the scanner's monitor or quantified by the post processing software is not a simple image of the dye fluorescence in the microarray's dots. It is the superposition of several images, only one of which is desired:

(a) The fluorescence of the target dye being scanned (which is desired).
(b) Photon statistical noise (really part of the image, but looks like noise).
(c) Fluorescence of the background, due to other chemicals and the glass (not desired).
(d) Laser light reflection (actually a reflection image of the sample, but not desired).
(e) Electronic noise (not desired).

One cannot determine the relative contributions of each by examining any one image or image data set by itself. The data set is just a collection of pixel brightnesses, and the A/D converter doesn't care what the source was. In evaluating a new instrument, a user needs to make sure that the instrument and the microarray preparation process work together to reveal results with the required accuracy.

8. ScanArray® confocal microarray scanners

GSI Lumonics has developed a product line of confocal microarray scanners called ScanArray®. As of this writing, hundreds of these instruments are in use in biotech and genomics laboratories around the world.

8.1 Importance of integration

The information provided in the above sections is sufficient to allow an experienced instrumentation engineer to build a breadboard microarray scanner. In fact, many laboratories have done just that, and many of those have published their instrument descriptions. However, a great deal of the value of a microarray scanner lies in its integration into a complete, compact, robust, reliable product. Most researchers don't want to build a complex instrument, they want to use it with confidence and not think about it. Commercial instruments, developed with the benefit of the user feedback that is required to

develop image quality standards and software control features, best meet researchers' needs.

8.2 ScanArray® architecture

The ScanArray® is a bench-top, moving-substrate, stationary optics, multi-laser, confocal scanner. This architecture utilizes simple optics, has a large NA for high light collection efficiency, and is low in cost. The compact size conserves valuable laboratory space. To benefit from those features without sacrificing throughput, a great deal of development effort has resulted in a servo-controlled sample moving mechanism that can scan up to 20 lines/sec.

HeNe and argon lasers of various colours provide excitation. A geometric beamsplitter and specially designed emission filters minimize laser reflection. A single PMT detector allows scanning one colour at a time, but with minimum cross-talk.

User control is provided by a host PC, either dedicated to the instrument in the ScanArray® 3000 or via a network connection in the ScanArray® 5000. Running under Windows®-95, 98, or NT, the host software provides quick, intuitive operation with convenience and automation of many tasks. *Table 2*

Table 2. ScanArray® specifications

	ScanArray® 3000 series	**ScanArray® 5000 series**
Architecture	Moving substrate, epi-illuminated, stationary optics, multicolour, sequential scanning	Moving substrate, epi-illuminated, stationary optics, multicolour, sequential scanning
Numerical aperture (NA)	0.75	0.75
Substrate	All 'standard' 1″ × 3″ microscope slides	All 'standard' 1″ × 3″ microscope slides
Field size	22 mm × 60 mm max. or smaller	22 mm × 73 mm max. or smaller
Image uniformity	± 10% max. over full field	± 10% max. over full field
Scanning speed	6 lines/sec max. at full field	20 lines/sec. max at full field
Resolution settings	10 μm; 50 μm (preview scan)	5, 10, 20 μm; 30, 50 μm (preview scans)
Lasers	Two (543 and 633 nm)	Up to four (488, 514, 543, 594, 612, or 633 nm)
Emission channels	2	Up to 10
Host computer	Dedicated Pentium®/Windows® PC	Networked Pentium®/Windows® PC
Output data	16-bit TIFF (.tif), bitmap (.bmp)	16-bit TIFF (.tif), bitmap (.bmp)
Dimensions	25″ long × 10.5″ wide × 11.5″ high	30″ long × 15.5″ wide × 13.5″ high
Power input	95–250 VAC, 47–63 Hz	95–250 VAC, 47–63 Hz
Weight	35 lb	60–70 lb, depending on laser configuration

illustrates the most significant specifications. Detectivity and cross-talk are not specified here as they are functional specifications that depend upon the substrate being scanned, for which commercial standards don't exist as of this writing. Suitability for an application is best determined by a demonstration.

8.3 ScanArray® advantages

Compared to the other commercially available microarray scanners, the ScanArray® product exhibits several advantages. The high NA objective lens combined with application developed filtering provides detectivity that leads the industry. The simple optical architecture leads to a very compact

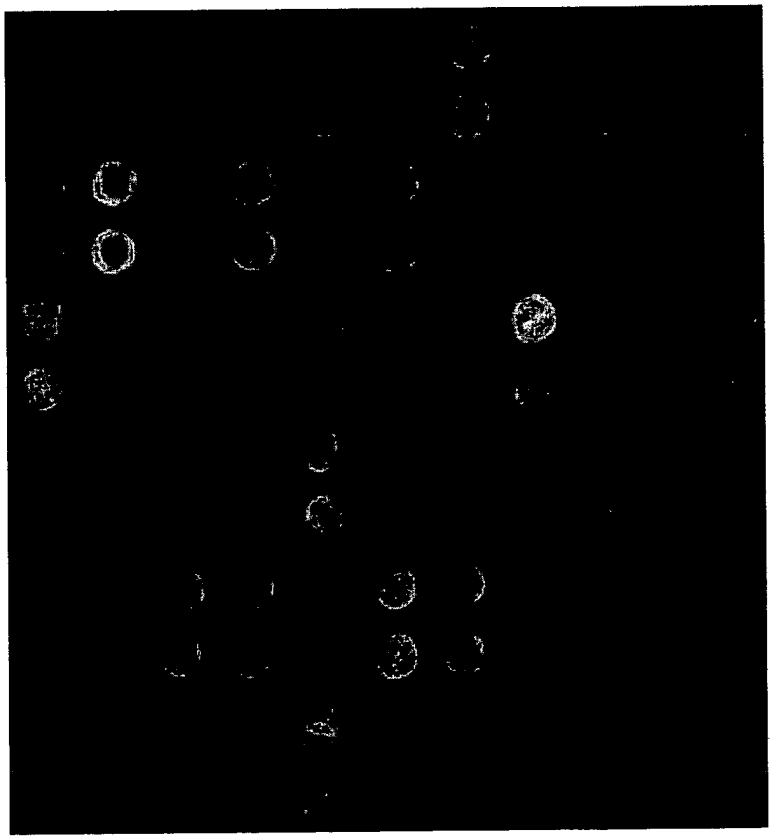

Figure 7. A small section of a fluorescence image from a gene expression array tagged with Cy3™. The dots are nominally 200 μm in diameter on 350 μm centre-to-centre spacing. This black and white representation does not reveal the range of fluorescent intensities to the eye as well as a false-colour image. Note the non-uniformity of intensities within some of the dots. Microarray quantification software will process this raw image data and produce a single value for each dot's intensity and local background value. This image was scanned by a ScanArray® 3000 instrument.

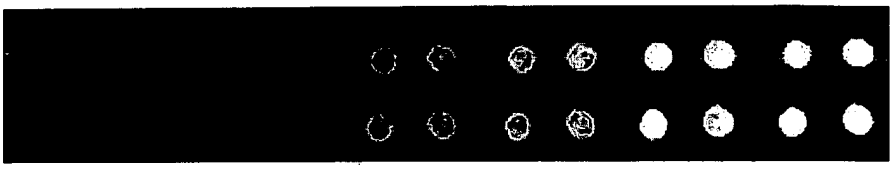

Figure 8. An example of a FITC dilution series used to test detectivity. Each group of four dots is a factor of four lower in concentration, moving right to left.

bench-top instrument with high reliability and low cost. Continuous collaboration with instrument users has resulted in a control interface that maximizes productivity with little training.

Microarray scanning instruments are complicated to compare. Many of the performance measures are interrelated and the selection criteria cannot be realistically narrowed down to a single specification; the entire instrument must be evaluated, often with a trial scan of the target samples. The specifications and features listed here were correct as of the time of writing. Features and upgrades are added periodically, so this description may not be current. *Figures 7* and *8* depict some greyscale microarray scanning results from ScanArray® instruments.

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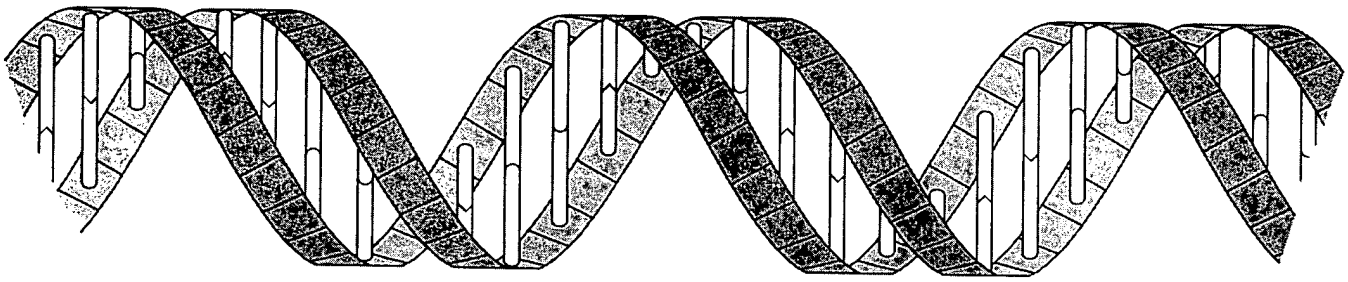
MICROARRAY ANALYSIS

Mark Schena, Ph.D.



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ink jetting, scanning, and imaging. Methodological principles (see Chapter 10) describe what needs to be done and why, rather than suggesting how the aforementioned will be accomplished. The epistemological distinction between methodology and method is significant, and students should endeavor to understand it. Methodology maintains the integrity of microarray analysis without stifling technology innovation. Microarray methods and tools have evolved immensely since the first microarray paper was published (Schena et al., 1995), and this evolution is likely to continue at a brisk pace as long as the toolmakers adhere to the general criteria put forth for microarray formats.

Microscopic Elements

Microscopic is defined as any object that cannot be seen clearly without the use of a microscope, which is nominally anything smaller than about 1 mm (1000 μm). Microarrays made by photolithography and the other semiconductor-based strategies typically produce 15- to 30-μm features, and printed microarray spot size is generally 50–350 μm. Most tissue microarrays contain spots of 200–600 μm, though the tissue microarray format is likely to shrink as the technology improves. The use of submillimeter microscopic elements is a clear departure from the earlier glass- and filter-based methods (see below), which used large printed elements > 1 mm in diameter. To qualify as a microarray element, the element must be smaller than 1.0 mm.

Microscopic. *An object that cannot be seen clearly without the use of a microscope and is measured typically in microns (1000 microns = 1 mm).*

Microarray elements are collections of **target** molecules that allow specific binding of **probe** molecules including genes and gene products, and a typical printed DNA spot contains approximately 1 billion (10^9) molecules attached to the glass substrate (see Appendix H). Microarray target material can be derived from whole genes or parts of genes, and may include genomic DNA, cDNA, mRNA, protein, small molecules, tissues, or any other type of molecule that allows quantitative gene analysis. Target molecules include natural and synthetic derivatives obtained from a variety of sources, such as cells, enzymatic reactions, and machines that carry out chemical synthesis. Synthetic oligonucleotides, short single-stranded molecules made by chemical synthesis, provide an excellent source of target material (see Chapter 6).

Target. *Molecule tethered to a microarray substrate that reacts with a complementary probe molecule in solution*

Probe. *Labeled molecule in solution that reacts with a complementary target molecule on the substrate*

What is the advantage of having microscopic elements? Small features or spots enable high density (>5000 elements/cm^2), rapid reaction kinetics and the analysis of entire genomes on a single chip. Experiments that examine all of the genes in the genome provide a comprehensive of a biological phenomenon (see Chapter 12) that is not possible with technologies limited to gene subsets. Microscopic spots enable miniaturization and automation, two key features of microarrays and microprocessors (see below). Filter arrays and other nonmicroarray formats made with large elements prevent miniaturization and automation and do not allow whole genome analysis in a miniature format.

Planar Substrate

A ***planar*** substrate is parallel and unbending support—such as glass, plastic, or silicon—onto which a microarray is configured. Glass is the most widely used substrate material owing to the many advantages offered by silicon dioxide (see Chapter 5), though other planar materials also work well. It is important to note that the requirement for a planar substrate is somewhat more stringent than the specification of a solid support. Glass, plastic, and silicon are solids but so

Planar. *Evaluative criterion for a microarray substrate that refers to the parallelism of the surface over the entire substrate.*